Microbial Characterization of Heat Melt Compaction for Treatment of Space Generated Solid Wastes

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One treatment process in development for solid waste management in space has been the Trash Compaction Processing System (TCPS). Human space mission wastes typically contain large percentages of contaminated wet solid waste. The Heat Melt Compactor (HMC) is being developed to be a multi-function means of water recovery, volume reduction, and the making safe of contaminant-rich trash with the potential for waste stabilization and/or sterilization. To determine the efficacy of the HMC treatment to kill microorganisms in solid waste and remain biologically stable, testing was conducted on three tiles produced by HMC-Gen 2 at Ames Research Center. Samples were shipped to Kennedy Space Center to test for microbial viability after compaction, determine the bio-stability of the HMC disks during storage (43 days), and assess potential airborne contaminate microbial growth on surfaces at low and high humidity conditions. In addition to the products of solid waste processing technologies, there is a concern that the crew might come into contact with hardware surfaces that have been contaminated by microorganisms during waste processing. The extent of microbial surface contamination of waste processing hardware was determined by surface sample swabbing and analysis for total bacterial and yeast counts and cultivable counts of aerobic and anaerobic bacteria, spore-forming bacteria, and fungi. Results indicate that trash processing increased bacterial counts on the surfaces of the compacter. All but one biological indicator spore strip imbedded in the HMC produced tiles were negative for growth after incubation for five days indicating effective sterilization through the heat melt compaction process. Analysis of core samples as well as surface growth of tiles inoculated with *Aspergillus niger* fungal spores incubated at three levels of humidity indicate that HMC created tiles did not support the proliferation of bacterial and fungal growth.

Nomenclature

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°C	=	Celsius	*IMA*	=	Inhibitory Mold Agar
AO	=	Acridine Orange	IMA	=	Inhibitory Mold Agar
AODC	=	Total Direct Count	KSC	=	Kennedy Space Center
ARC	=	Ames Research Center	mL	=	milliliters
BI	=	Biological Indicator	PDA	=	Potato Dextrose Agar
CFU	=	Colony Forming Units	RH	=	Relative Humidity
DI	=	deionized water	TCPS	=	Trash Compaction Processing System
ETO	=	Ethylene Oxide	TNTC	=	Too numerous to count
FY	=	Fiscal Year	*TSA*	=	Trypticase Soy Agar
Gen 2	=	Generation 2 Heat Melt Compactor	µL	=	microliters
HMC	=	Heat Melt Compactor	µm	=	micrometers
HS	=	Heat Shock			

I. Introduction

One treatment process in development for solid waste management in space has been the Heat Melt Compaction (HMC) technology, which is operated to reduce trash volume by compaction and heat, while simultaneously removing water[1,2]. Human space mission wastes typically contain large percentages of plastic contaminated with wet waste[2]. The Gen 2 HMC is being developed as a risk reduction laboratory hardware similar in function to the Trash Compaction Processing System (TCPS) to be a multi-function means of water recovery and volume reduction of plastic rich trash with the potential for waste stabilization and/or sterilization[2].

Microbial characterization studies of space generated solid wastes have been done at Kennedy Space Center (KSC) since 2007[3,4,5] . Earlier studies showed that simulated Space Shuttle mission trash supported the growth of human associated pathogens over long periods (6 weeks) of storage. Results from the analysis of Space Shuttle trash in which potential human pathogens were identified demonstrate the potential problems regarding pathogens as cross-contaminants when handling such material[5]

By removing the water, a valuable recoverable resource, the waste is stabilized biologically and less likely to support the growth of microbes including potentially pathogenic ones. In this process, waste is exposed to temperatures that are high enough to be lethal to microorganisms and over a long enough time to potentially disinfect the waste and yield a product that is low in volume and mass[2].

The HMC project is aimed at the development of a volume reduction system that has a lower Equivalent System Mass (a sum of weight, power, volume, and crew time) than systems that store waste in a hand-compacted state. The current focus is on compactors that can have application to both short and long duration missions. To determine the efficacy of heat melt compaction treatment to kill microorganisms in solid waste and remain biologically stable the following tasks were completed:

1) ***Microbiological analysis of HMC hardware surfaces before and after operation.*** In addition to the actual products of the solid waste processing technologies, there is a concern that crew might come into contact with hardware surfaces that have been contaminated by microorganisms during waste processing. The objective of this task was to determine the extent of microbial surface contamination on the waste processing hardware. Samples were analyzed for total bacterial and yeast counts and cultivable counts of aerobic and anaerobic bacteria, spore-forming bacteria, and fungi.
2) ***Microbiological analysis to determine the effectiveness of the HMC for reducing the microbial load of solid waste(s).*** To test for microbial viability after compaction, biological indicator (BI) spore strips (Namsa, Northwood, Ohio) of *Bacillus atrophaeus* and *Geobacillus stearothermophilis* were strategically placed in the trash before compacting. After the compacted tiles were made, they were sealed in a plastic bag and shipped to KSC for analysis.
3) ***Assessment of subsequent microbial proliferation on HMC tile surfaces (the high liquid TCPS trash model) after reintroduction of microorganisms at three different humidity levels.*** To determine the bio-stability of the HMC disks during storage (43 days), and potential airborne contaminate microbial growth on surfaces, controlled experiments were performed at three different relative humidity levels.

II. Materials and Methods

A. Microbiological analysis of the HMC hardware surfaces through swab sampling.

Sterile Sanicult swabs (Starplex Scientific, Cleveland, TN, USA) were used at Ames Research Center (ARC) to perform surface samples of the HMC hardware before and after use (Figure 1). After sampling, the swabs were shipped on ice, overnight to KSC for microbiological analysis on the day of receipt if possible or held at 4° C until processing.

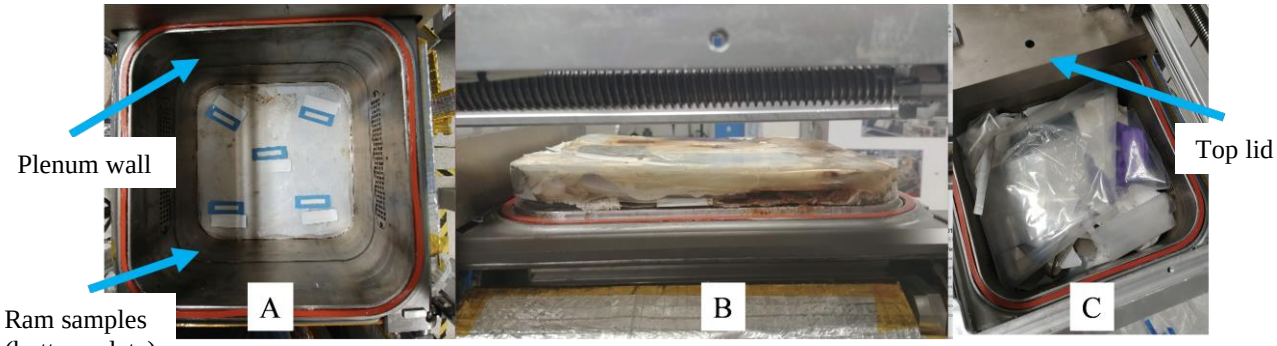

Figure 1. HMC Gen 2 chamber: (A) and (B) inner chamber showing the locations of the plenum and Ram. (A) Ram is the bottom plate that moves up as the trash is compacted. Plenum samples are taken from the inner walls; (B) after processing, the ram is fully extended "up" for tile removal. Here, swab samples were taken on the aluminum ram plate after the trash tile is removed, on the surface below the trash disk. (C) chamber with trash showing the top lid. This lid will slide close before operation. Teflon sheets were placed both on the lid and ram to prevent the finished tile from adhering to both the ram and the lid after compaction.

Sanicult swabs were contained in a tube containing 5 milliliters (mL) sterile buffer. Each tube was vortex mixed for 30 seconds to remove microorganisms from the sampling swab for processing. The following analyses were performed on the buffer and subsequent samples.

1. *Total Direct Count (AODC)*[6]. One mL samples were stained with 100 µL of Acridine Orange (AO) and filtered through a black polycarbonate filter (Nuclepore, Whatman,) with a 0.2 µm pore size. Filters were placed on a microscope slide and direct microscopic counts were done using a Zeiss Epi-Fluorescent Axioskop microscope at 1000 X magnification. Microscope software used for counting and observing the cells is DP Manager, DP Controller, and Image-Pro Express 6.3.
2. *Cultivation based enumeration of bacteria and fungi.*

Buffer solution (see above) was directly plated onto agar growth media or diluted if necessary before plating. Different groups of bacteria listed below were selectively grown based on incubation atmosphere and/or heat treatment and enumerated following incubation (48 hrs-1 week):

a) Aerobic, mesophilic bacteria. Sample was spread onto trypticase soy agar (TSA) (Thermo Fisher Scientific, Waltham, MA, United States) and incubated aerobically at 30 °C.
b) Anaerobic, mesophilic bacteria. Sample was spread onto TSA, incubated anaerobically, (plates were placed in an anaerobe box with an anaerobic atmosphere generating sachet) at 30 °C.
c) Aerobic sporeformers. The liquid sample was heat treated by incubation for 15 minutes in a heat block set at 80 °C. The sample was allowed to cool to room temperature, plated onto TSA and incubated at 30 °C.
d) Anaerobic sporeformers. Same method as above (aerobic) except the plates were incubated anaerobically.
e) Fungi (yeasts and molds). Samples were plated onto Inhibitory Mold Agar (IMA) (Thermo Fisher Scientific, Waltham, MA, United States) and incubated at 30 °C for between 48 hrs to 1 week.

3. *Identification of isolated microbes.*

Following colony enumeration, individual colony phenotypes were picked and streaked for isolation on appropriate growth medium and incubated. Isolated bacterial colonies were identified using the Micro Id System (Biolog, Hayward, CA, United States) or MicroSEQ 16S rDNA sequencing kit for bacteria (Thermo Fisher Scientific,

Waltham, MA, United States). Fungal colonies were identified using the MicroSEQ D2 LSU rDNA kit for fungi (Thermo Fisher Scientific, Waltham, MA, United States). Sequencing was completed on the ABI 3130 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, United States). Bacterial and fungal DNA sequences were identified using MicroSEQ ID Software V2.0 (Bacterial Library, 2009; Fungal Library, 2011, Applied Biosystems, Life Technologies, Foster City, CA, United States) and/or NCBI Basic Local Alignment Search Tool (BLAST).

B. Microbiological analysis to determine the effectiveness of the HMC for reducing the microbial load of solid waste(s).

Figure 2. Tile created from the HMC process. Note the sterilization indicator spore strips before removal.

Biological indicator (BI) spore strips close to the surface (Figure 2) were excised from the tiles before core sampling using sterile scalpel and forceps. Strips were processed and incubated according to the manufacturer's protocol. Disk surfaces and three core samples per ¼ section of the tile were processed (Figure 3). The entire surface of the tile was swabbed before and after sanitizing the tile with 70% ethanol. The sanitizing step was performed to minimize contamination of the core samples. This served as the Time=0 sample for comparison with the time course samples incubated at different humidity levels for 43 days.

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Figure 3. Core sampling of ¼ section of HMC tile for micro processing.

Core samples (Figure 3) were suspended in 10-20 mL of sterile deionized (DI) water containing sterile glass beads. These were shaken vigorously for 30 seconds to remove bacteria and fungi from the surfaces. These samples were processed for microbial characterization as per the methods described in microbiological analysis for enumeration of aerobic and anaerobic bacteria, gram-positive spore forming bacteria, and fungi. After colony enumeration, bacteria and fungi were streaked for isolation on a general growth media like TSA or TSA for bacteria and IMA or Potato Dextrose Agar (PDA) for fungi and incubated for 24-48 hours. Isolates were identified first by the Biolog Micro-ID system (Biolog, Hayward, CA) following manufacturer's protocols. If the Biolog system failed to identify the isolate, then the Micro-Seq method was used following manufacturer's protocols.

C. Long term storage at three different humidity levels.

1. Inoculation of surfaces.

After surface sampling, each tile was cut into four equal sections using tools and gloves sanitized with ethanol under a biological safety cabinet aseptically to minimize contamination. One quarter section from each tile was sampled immediately. The remaining three sections were placed in the incubation chambers (Figure 5) to be sampled over time: 14 days, 29 days, and 43 days. These sections were also inoculated with a pre-determined number of

Aspergillus niger spores. Three core samples, 1.25 cm (1/2 inch) in diameter were removed from the disk section using a hand press with a sharpened hole punch.

For inoculation of the surfaces with fungal spores, *Aspergillus niger* was grown on IMA plates to produce spores. Spores were harvested by flooding the surface of the plates with sterile phosphate buffered saline plus 0.3% Tween 80 then scrapping them gently off the surface and pipetting into a sterile tube. Spores were enumerated under a microscope using a Neubauer hemocytometer slide. A spore suspension of approximately 1000 spores/mL was inoculated onto the disk surface in a delineated 2 x 2 cm^2 area on each ¼ section and allowed to dry (Figure 4).

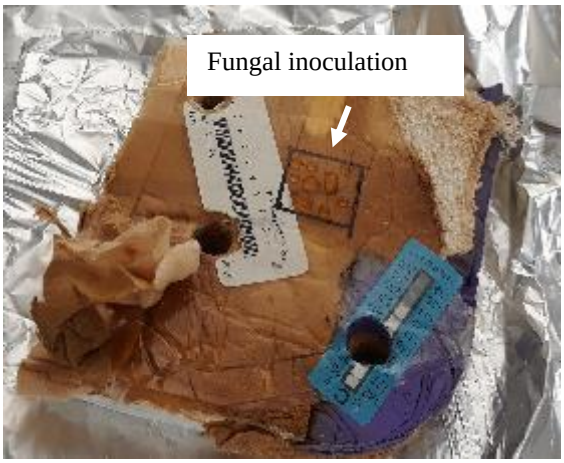

Figure 4. HMC Gen 2 tile section after core samples and fungal inoculation

4. Incubation of HMC disks and microbiological analysis.

Four time points or storage durations, 0, 14, 29 and 43 days at three different humidity levels were tested. The humidity level on the International Space Station (ISS) is usually around 50% or lower, so the humidity levels used bracketed around those conditions. Each chamber (black acrylic, 11 in x 11 in x 12 in) (Figure 5) maintained a different humidity environment created by including small containers of saturated solutions with the following compounds within the chambers:

Ammonium sulfate - $(NH_4)_2SO_4$	RH 81% @ 20 °C
Magnesium nitrate - $Mg(NO_3)_2$	RH 54% @ 20 °C
Potassium acetate - CH_3CO_2K	RH 23% @ 20 °C

Temperature and humidity were monitored by sensors and recorded using Opto 22 temperature and RH input modules using an existing Opto 22 data logging system. Table 3 shows the average temperature and relative humidity readings for each chamber including the tile incubation period in July and August 2021.

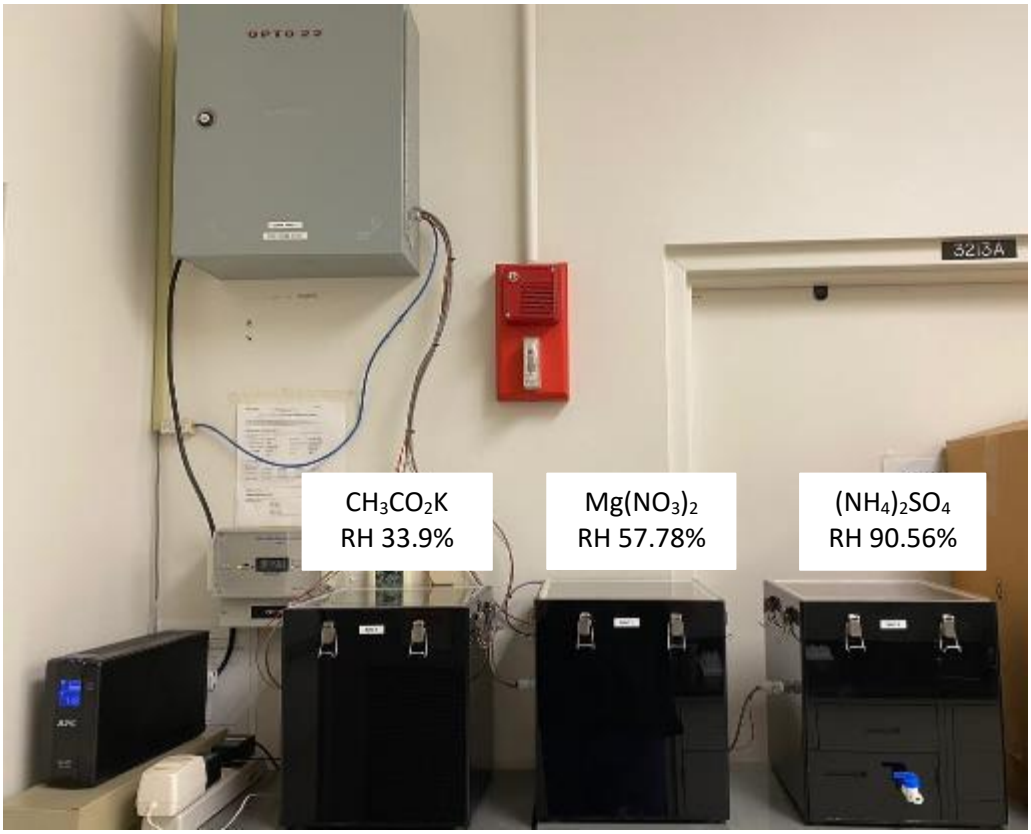

Figure 5. Humidity chambers connected to Opto 22 box for monitoring RH and Temperature.

Table 1. Average data collected by Opto 22 from the humidity chambers over a three-month period. Data are logged every five minutes.

HMC Data Averaged							
Month	**Laboratory Temp**	**Temp1**	**RH1**	**Temp2**	**RH2**	**Temp3**	**RH3**
June	23.5	23.2	34.9	23.1	56.0	23.4	89.0
July	23.6	22.9	33.6	23.0	58.3	23.2	92.2
August	23.6	22.9	33.4	22.9	59.0	23.1	90.4
	23.6	**23.0**	**34.0**	**23.0**	**57.8**	**23.2**	**90.5**

Samples were removed after 14, 29, and 43 days of incubation for microbiological analysis. Surface samples were taken using swabs on the pre-inoculated sections to enumerate *Aspergillus niger* spores. Three core samples were taken from each section and processed for microbiological counts as described previously in this paper.

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III. Results

D. HMC Surface Swab samples.

Samples were taken of surfaces from the HMC before (initial) and after (final) use. The aerobic/ anaerobic bacterial counts were higher from the final samples, except for the lid location, while the fungal counts were lower. Lower fungal count could be attributed to the heaters mounted on the lid. These data indicate that trash processing increased bacterial counts on the surfaces of the compactor (Table 2).

Table 2. Bacterial (aerobic and anaerobic) and fungal counts on surfaces (CFU/swab). HS= heat shock for selection of spore formers. Numbers in red indicate the detection limit for specific tests. Sample descriptions are as follows: plenums refer to the side walls, ram refers to the bottom plate inside the chamber, and lid refers to the top lid.

Sample location	Aerobic	HS-Aerobic	Anaerobic	HS-Anaerobic	Fungi	Direct counts
Plenums initial	≤1.6E+00	≤2.0E+00	≤1.3E+01	≤2.5E+01	≤3.1E+00	≤4.0E+03
Wall initial	1.6E+03	≤2.0E+00	≤2.5E+01	≤2.5E+01	2.5E+01	≤4.0E+03
Ram initial	3.3E+06*	6.8E+02	3.3E+05*	≤2.5E+01	3.8E+02	2.4E+07
Lid initial	3.9E+03	≤2.0E+00	≤1.0E+00	≤2.5E+01	≤3.1E+00	≤4.0E+03
Plenums final	3.5E+02	≤2.0E+00	≤1.0E+00	≤2.5E+01	≤3.1E+00	5.2E+04
Wall final	3.3E+06*	2.1E+04	3.3E+05*	3.3E+05*	≤3.1E+00	8.8E+06
Ram final	3.3E+06*	1.5E+03	3.3E+05*	3.3E+05*	2.5E+01	2.4E+06
Lid final	8.1E+02	1.0E+02	≤5.0E+01	≤2.5E+01	≤3.1E+00	2.0E+05

*estimates from plates with high colony number or too numerous to count (TNTC)

E. Bioindicator spore strips

All spore strips were negative for growth after incubation for five days indicating effective sterilization through the HMC process, except for the number 3 tile *B. atrophaeus* spore strip. This strip could have been in a location that did not receive the exposure time and temperature necessary for sterilization. The *Geobacillus geothermopilus* strips from tile 3 yielded negative results indicating effective temperature and time exposure to achieve surface sterilization. This bioindicator is specifically used for validating wet heat sterilization processes, while the *B. atrophaeus* is an indicator used for lower heat processes such as ETO sterilization and dry heat.

F. Core samples

After tiles were cut into ¼ pieces, three core samples were taken from each quarter at each sample time point and analyzed for microbial counts (Table 2). At T=0, all samples were below the detection limit in the low humidity (tile 3). Tile 3 counts continued to be below detection for the duration of the 43-day incubation period except one sample at T=day 29. The medium humidity incubated tile (Tile 4), started with an average aerobic bacterial count of 3.3 CFU/core sample. This increased to 50 CFU/core at day 29, but at day 43 the counts were below detection. Tile 5, which was incubated at the high humidity, yielded core sample average counts for aerobic bacteria (30 CFU), spore formers (13.3 CFU), anaerobic bacteria (13.3 CFU) and anaerobic spore formers (26.7 CFU) at T=0. No fungi were detected. At Day 14, the aerobic and anaerobic bacterial counts increased to 75 and 160 CFUs/core respectively but declined to 2 CFU/core and below detection limit by day 43. Aerobic and spore forming bacteria in tile 4 incubated in the medium humidity range, increased slightly after 29 days of incubation, but after 43 days, declined to below detection limit.

Table 3. Average counts (CFU/core) of aerobic and anaerobic bacteria from core samples from tiles incubated at three different humidity levels for 43 days. HS= heat shocked samples selecting for spore forming bacteria.

Days	Humidity (tile)	Aerobic	HS-Aerobic	Anaerobic	HS-Anaerobic	Fungi
T=0	Low (3)	<DL	<DL	<DL	<DL	<DL
	Medium (4)	3.3	13.3	0.0	20.0	<DL
	High (5)	30.0	13.3	13.3	26.7	<DL
T=14	Low (3)	<DL	<DL	<DL	<DL	<DL
	Medium (4)	10.0	10.0	<DL	<DL	<DL
	High (5)	75.0	<DL	160.0	<DL	<DL
T-29	Low (3)	45	<DL	5	<DL	<DL
	Medium (4)	50	5	<DL	<DL	5
	High (5)	15	<DL	20	<DL	<DL
T-43	Low (3)	<DL	<DL	<DL	<DL	<DL
	Medium (4)	<DL	<DL	<DL	<DL	<DL
	High (5)	2	<DL	<DL	<DL	4

Ten bacterial genera were isolated and identified from all core samples and are listed below:

1. *Bacillus pumilus/safenisis-*
2. *Stenotrophomonas maltophilia*
3. *Pseudomonas oryzihabitans*
4. *Staphylococcus epidermidus*
5. *Staphylococcus capitis ss capitis*
6. *Staphylococcus xylosus*
7. *Paenibacillus harenae*
8. *Paenibacillus barainonensis*
9. *Paenibacillus pabuli/taichungensis*
10. *Paenibacillus xylanilyticus*

1.4. Aspergillus niger surface growth.

At T=0, 2 cm^2 surfaces were inoculated with approximately 1000 spores of *A. niger*. The counts from the low humidity incubated tiles were 60 CFU/ cm^2 after 14 days, however after 28 days the spore level was below detection. In the case of the medium and high humidity incubated tiles, at 14 days and after, no fungal growth was detected. These results indicate that the HMC tiles did not support the proliferation of fungal growth on the surfaces.

IV. Conclusion

Microbiological testing indicates that the heat melt compaction process is effective in reducing the microbial load in trash and sterilizing the internal surfaces of the unit itself in the process. Microbial counts were low to below detection limits from core samples taken from the tiles. Sections of the tiles were incubated in three humidity levels, low, medium and high to ascertain the possibility of growth of fungal contamination on the tile surface and growth of internal microorganisms. Testing showed that after intentional *A. niger* fungal inoculation on the surface, fungal counts decreased to very low to below detection after 14 days of incubation and after 28 days all were below detection at all humidity levels. Several bacteria were isolated and identified from the core samples. Many of these are aerobic spore formers (*Bacillus and Paenibacillus*), more resistant to heat than non-spore forming bacteria. The *Staphyloccocus* species isolated are common in the environment and on human skin. The surfaces of the tiles could have been

contaminated post treatment as a result of handling. None of the organisms isolated are usually associated with human disease.

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